An Improved Synthesis of 3-Deazacytosine, 3-Deazauracil, 3-Deazacytidine, and 3-Deazauridine

P. Dan Cook (1a), Robert T. Day (1b), and Roland K. Robins (1c)

Contribution from ICN Pharmaceuticals, Inc., Nucleic Acid Research Institute, Irvine, California 92715

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3-Deazacytosine (4-amino-2-pyridone, **3**), 3-deazauracil (4-hydroxy-2-pyridone, **5**), 3-deazacytidine (4-amino-1-β-D-ribofuranosyl-2-pyridone, **9**), and 3-deazauridine (4-hydroxy-1-β-D-ribofuranosyl-2-pyridone, **11**) were prepared in high overall yields from 1-methoxy-1-buten-3-yne (**1**). Ethyl 3,5,5-triethoxy-3-pentenoate (**2**), obtained from acylation of **1** with diethyl carbonate and subsequent *in situ* conjugate addition of ethoxide, was cyclized with ammonia to provide **3**. Diazotization of **3** and subsequent *in situ* hydroxydediazotization afforded **5**. Nucleoside **9** was obtained from the stannic chloride-catalyzed condensation of bis-trimethylsilylated **3** and 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose (**7**), followed by ammonolysis of the blocking groups. Diazotization of **9** and subsequent *in situ* hydroxydediazotization afforded nucleoside **11**.

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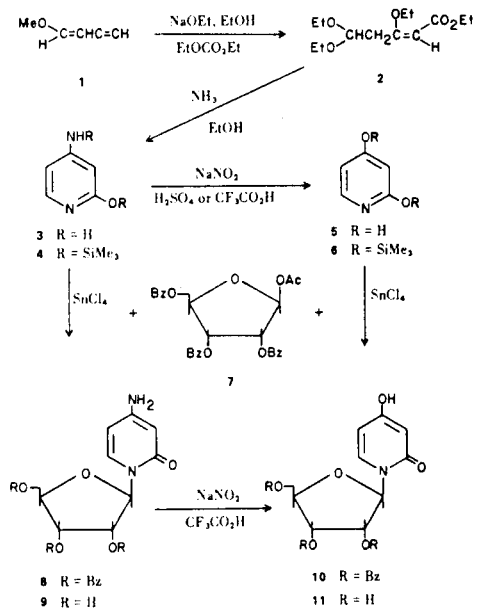
3-Deazacytidine (4-amino-1-β-D-ribofuranosyl-2-pyridone, **9**) and 3-deazauridine (4-hydroxy-1-β-D-ribofuranosyl-2-pyridone, **11**) (2), the pyridine analogs of the naturally occurring pyrimidine nucleosides, cytidine and uridine, are the most biologically interesting compounds to be derived from this type of structural modification (3-deazapyrimidine nucleosides) (3,4). 3-Deazacytidine and 3-deazauridine are effective against tumor growth *in vitro* and *in vivo*, possess moderate activity against a number of bacterial systems *in vitro* (4d), and have a wide spectrum of anti-RNA viral activity in various cell cultures (5). Biochemical and mechanism of action studies of 3-deazacytidine and 3-deazauridine have been reported (6). However, the most recent and interesting disclosures concerning these nucleoside analogs lie in the effectiveness of 3-deazauridine on cutaneous lesions in patients with nodular basal cell carcinomas (7) and activity *in vitro* against neoplastic hepatic cells (8a). 3-Deazauridine (**11**) is currently undergoing clinical trial as an anticancer agent (8b). Although these nucleosides (**9** and **11**) possess a wide range of interesting biological activities which have great potential chemotherapeutic uses, a practical synthesis of these compounds has not been reported.
prepared by Currie *et al.* (2) by ribosylation of 4-acetamido-2-methoxypyridine and 3-deazauracil (**5**), respectively. Although these were high-yield ribosylations, the low yield of the synthesis of the requisite bases renders the synthesis of 3-deazacytidine (**9**) and 3-deazauridine (**11**) impractical (9).

We now report a simple, high-yield (84%), two-step synthesis of 3-deazacytosine (**3**) from the inexpensive, readily available 1-methoxy-1-buten-3-yne (**1**). An additional reaction (hydroxy-dediazotization, 93%) affords 3-deazauracil in an overall yield of 78%.

3-Deazacytosine (**3**) was first prepared by a lengthy synthesis from 2-chloroisonicotinic acid (10). Another lengthy, low-yield procedure from 2-chloropyridine was recently described by Currie *et al.*, (4d). Our new approach utilizes ethyl 3,5,5-triethoxy-3-pentenoate (**2**) obtained in 90% yield from the acylation of **1** with ethyl carbonate in ethanolic-sodium ethoxide and subsequent *in situ* conjugate addition of ethoxide (11). Cyclization of **2** with methanolic ammonia and ammonium chloride and subsequent *in situ* amino-deethoxylation provided 3-deazacytosine (**3**) in 93% yield (12). Talik and Talik (13) attempted to diazotize **3** with sodium nitrite in dilute sulfuric acid, but preferential nitrosation in the 3-position was obtained (13). We have diazotized **3** by use of anhydrous conditions (sodium nitrite in concentrated sulfuric acid or trifluoroacetic acid). Subsequent *in situ* hydroxy-dediazotization proceeded rapidly to form 3-deazauracil (**5**) in 93% yield. Schroeder and Rigby (14) have reported a similar reaction in which 4-amino-2-hydroxy-6,7-dihydro-5*H*-pyrindine was diazotized followed by *in situ* hydroxy-dediazotization. The use of dilute hydrochloric acid provided the 4-amino-2-hydroxy-3-nitroso-6,7-dihydro-5*H*-pyrindine (14).

Direct ribosylation of bis-silyl-3-deazacytosine (**4**) with 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribose (**7**) in the presence of one molar equivalent of anhydrous stannic chloride provided 4-amino-1-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)-2-pyridone (**8**) in 97% yield. This ribosylation procedure, first described by Vorbrüggen (15) and subsequently employed in these Laboratories with excellent results (16), has the advantage of reacting the readily available acylated ribofuranose **7** with the unacylated amino heterocycle **3** rather than reacting the 2,3,5-tri-*O*-benzoyl-D-ribofuranosyl bromide with 4-acetamido-2-methoxypyridine in a modified Hilbert-Johnson reaction as in the original procedure of Currie, *et al.*, (2b). Methanolic ammonia treatment of blocked nucleoside **8** provided 3-deazacytidine (**9**) in 86% yield (70% from 1-methoxy-1-butene-3-yne, **1**) without the need for ion-exchange purification (17). The diazotization of 3-deazacytidine (**9**) and subsequent *in situ* hydroxy-dediazotization to 3-deazauridine (**11**, 85%, 69% from 1-methoxy-1-butene-3-yne, **1**) was obtained with anhydrous trifluoroacetic acid and sodium nitrite. 3-Deazauridine (**11**) may also be obtained by the direct ribosylation of 3-deazauracil with a modified Hilbert-Johnson procedure, as



described by Currie *et al.*, (2b) or by the Vorbrüggen procedure utilizing anhydrous stannic chloride (15,18). These direct ribosylation procedures require basic conditions for the debenzoylation of **10** and desalting by acidic ion-exchange chromatography (17).

Thus, we have presented a short, simple, high-yield synthesis of 3-deazacytosine, 3-deazauracil, and their β-D-ribofuranosides from inexpensive, readily available materials.

EXPERIMENTAL

Melting points were determined with a Thomas-Hoover capillary melting point apparatus and are uncorrected. Specific rotations were measured in a 1-dm tube with a Perkin-Elmer Model 141 automatic digital readout polarimeter. Proton magnetic resonance (^{1}H nmr) spectra were obtained on a Varian A-60 spectrometer and a Perkin-Elmer R-20A spectrometer in DMSO-d_6 using DSS as an internal reference. Ultraviolet spectra were recorded on a Cary Model 15 spectrophotometer and infrared spectra on a Perkin-Elmer 257 spectrophotometer (potassium bromide pellets). Elemental analyses were performed by Galbraith Laboratories, Inc., Knoxville, Tenn. Evaporations were carried out under reduced pressure with bath temperature below 40° unless otherwise noted. Detection of components on silica gel (ICN Life Sciences Group, Woelm F254) was by ultraviolet light and with anisaldehyde, methanol, sulfuric acid (1:10:100) spray followed by heating. ICN Life Sciences Group Woelm silica gel (0.063-0.2 mm) was used for column chromatography.

1-Methoxy-1-butene-3-yne (**1**).

A 50% solution of 1-methoxy-1-butene-3-yne in aqueous methanol (19) (500 g.) was distilled at atmospheric pressure from a one-liter flask fitted with a 2.5 x 50-cm Vigreux column with a fraction-cutter take-off. The following fractions were obtained: (a) 225 ml. of mostly methanol (60-70°, oil bath 100-110°), (b) 150 ml. of *ca.* 1:1 mixture of **1** and water, (c) 140 ml. of **1** (122-125°, oil bath 160-170°). Fraction (b) was extracted with ether. The dried (magnesium sulfate) ether extract was distilled to provide 75 g. of **1**. Total yield of **1** as a light yellow oil was 202 g., b.p. 122-125° (760 torr); ^1H nmr (carbon tetrachloride): δ 2.81 (d, 1, J = 2.5 Hz, C≡C-*H*), 3.79 (s, 3, CH_3), 4.33 and 4.41 (dd, 1, J = 7.0 Hz, J = 2.5, C=C*H*-C≡C), 6.25 (d, 1, J = 7.0 Hz, CH_3OCH).

Anal. Calcd. for C_5H_6O (82.1): C, 73.1; H, 7.31. Found: C, 73.4; H, 7.43.

Ethyl 3,5,5-Triethoxy-3-pentenoate (**2**).

This procedure is a modification of Dornow and Ische's procedure (11). A solution of sodium ethoxide (1.46 moles, prepared from 33.6 g. of sodium and 700 ml. of absolute ethanol) and diethyl carbonate (190 g., 1.61 moles) was stirred rapidly as **1** (120 g., 1.46 moles) was added dropwise (*ca.* 1.5-2 hours). After this addition, the solution was kept at 70-80° for 3 hours, cooled to 5-10°, and neutralized with aqueous acetic acid [82.6 ml., 1.46 moles, and 300 ml. of water]. The reaction mixture was extracted with chloroform. The extracts were treated with charcoal and dried (magnesium sulfate). Removal of the chloroform *in vacuo* provided 342 g. (90%) of yellow oil. A sample of this material was distilled to afford a light yellow oil, b.p. 140-145° (10 torr) (Lit. b.p. 147°, 12 torr) (11). Tlc and pmr analysis indicated that the 342-g. sample (before distillation) and the distilled sample were identical; ^1H nmr (carbon tetrachloride): δ 1.03-1.47 (m, 12, CH_3), 3.00 (d, 2, J = 6.0 Hz, CH_2), 3.40-4.13 (m, 8, CH_2CH_3), 4.74 (t, 1, J = 6.0 Hz, C*H*), 4.91 (s, 1, C*H*-CO_2Et).

Anal. Calcd. for $C_{13}H_{24}O_5$ (260.3): C, 59.98; H, 9.29. Found: C, 60.33; H, 9.36.

4-Amino-2-pyridone (3-Deazacytosine, **3**).

A mixture of **2** (100 g., 385 mmoles), ammonium chloride (204 mg., 3.85 mmoles), and 250 ml. of ethanolic ammonia (saturated at -10°) in a 300-ml. steel bomb was stirred and heated (150-160°) for 10 hours. The solvents were removed *in vacuo* and the residue was recrystallized (charcoal) from methanol-ether to provide 39.4 g. (93%, in two crops) as light yellow needles (12), m.p. 220-221° (after drying at 100° for 5 hours) (Lit. m.p. 218.5-220° (4d), 219-221° (10)); λ max (*p*H 1) 253, 213 nm (ε, 15,950; 18,360); λ max (*p*H 7) 260, 267 sh (7,670; 7,410); λ max (*p*H 11) 260, 267 (7,920; 7,670); ^{1}H nmr (DMSO-d_6): δ 5.30 (d, 1, J = 1 Hz, C_3H), 5.68, 5.80 (dd, 1, J = 7.0 Hz, 1 Hz, C_5H), 6.18 (s, 2, NH_2), 7.09 (d, 1, J = 7.0 Hz, C_6H), 10.9 (bs, 1, N*H*).

Anal. Calcd. for $C_5H_6N_2O$ (110.1): C, 54.54; H, 5.49; N, 25.44. Found: C, 54.51; H, 5.44; N, 25.42.

4-Hydroxy-2-pyridone (3-Deazauracil, **5**).

Method A.

Powdered **3** (11.0 g., 100 mmoles) was dissolved with stirring and cooling (<30°) in concentrated sulfuric acid (83 ml.). Sodium nitrite (6.9 g., 100 mmoles) was added portion-wise with efficient stirring and cooling done to maintain the reaction temperature between 35-45°. After this addition, the reaction was stirred at 60° for 15 minutes, cooled, and poured into 100 g. of crushed ice. Boric acid (12.0 g., 194 mmoles) was added, and the solution was rapidly heated to 100° and then cooled to precipitate ~ 10 g. of salts. The filtrate was adjusted to *p*H 7 with concentrated ammonium hydroxide and evaporated *in vacuo* (60°) to dryness. The residue was triturated with hot methanol (500 ml., in several portions). The hot methanolic extracts were treated with charcoal, filtered, and evaporated to dryness *in vacuo* to afford 10.3 g. (93%) of fairly pure **5**. Recrystallization from methanol provided 9.9 g. (85% in two crops) of pure **5** as large colorless needles (12), m.p. 275-277° dec. (after drying at 100° for 5 hours) (Lit. 271-273°) (4d); λ max (*p*H 1): 275 sh, 259 223 nm (ε, 2760, 4280, 4400); λ max (*p*H 7): 264, 258 sh

(5,790; 5,530); λ max (*p*H 11): 259 sh, 254 (6410, 6530); ^{1}H nmr (DMSO-d_6): δ 5.61 (d, 1, J = 2.5 Hz, C_3H), 5.83, 5.96 (dd, 1, J = 7.5, 2.5 Hz, C_5H), 7.30 (d, 1, J = 7.5 Hz, C_6H), 11.13 (bs, 2, N*H*, O*H*).

Anal. Calcd. for $C_5H_5NO_2$ (111.1): C, 54.05; H, 4.54; N, 12.61. Found: C, 54.07; H, 4.57; N, 12.67.

Method B.

Powdered sodium nitrite (2.67 g., 40 mmoles) was added portion-wise to **3** (4.4 g., 40 mmoles) dissolved in anhydrous trifluoroacetic acid (60 ml.). Temperature was maintained between 40-45° with some ice-bath cooling during addition period (*ca.* 10 minutes). Immediate evolution of nitrogen was observed. After addition, the solution was heated at 50° for 15 minutes; then urea (250 mg.) was added and the solution evaporated *in vacuo* (finally at 50°, 0.1 torr). The residue was partly dissolved in methanol (150 ml.) and treated with Dowex 50W x 8 resin (hydrogen form, 30 ml. of methanol washed). After stirring for 15 minutes, the resin was filtered and washed with hot methanol. The methanol solution was treated with charcoal and rapidly passed through a short silica gel column (50 g.). Evaporation of the methanol provided 4.2 g. of off-white **3**. Tlc (silica gel, chloroform-methanol, 4:1; isopropyl alcohol-ammonia hydroxide-water, 7:1:2; or acetonitrile-0.2 *M* ammonium chloride, 3:1) indicated pure **3**.

Recrystallization from methanol-ether provided **3** (3.7 g., 83%) as colorless needles, m.p. 275-277° dec. (after drying at 100° for 5 hours).

4-Amino-1-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)-2-pyridone (**8**).

4-Amino-2-pyridone (**3**, 11.0 g., 100 mmoles) was refluxed and stirred under anhydrous conditions for 8 hours with hexamethyldisilazane (HMDS, 200 ml.) and ammonium sulfate (20 mg.). The excess HMDS was removed by distillation under reduced pressure providing the trimethylsilyl derivative as a brown oil. The oil was dissolved in dry 1,2-dichloroethane (200 ml.). 1-*O*-Acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose (**7**, 50.4 g., 100 mmoles) was added to the solution followed by direct addition of anhydrous stannic chloride (11.7 ml., 100 mmoles) in one portion. Tlc (silica gel, ethyl acetate) of a methanolyzed aliquot indicated almost complete conversion of base and sugar to one product after 0.5 hour stirring at ambient temperature. The dark reaction solution was stirred at ambient temperature for 8 hours and then poured slowly into a vigorously stirred 5% aqueous sodium hydrogen carbonate solution (750 ml.). Chloroform (1 l.) was added, and after 15 minutes of stirring the suspension was filtered through a bed of celite. The organic layer was separated, dried (magnesium sulfate), and evaporated *in vacuo* to a yellow foam (54 g., 97%). This material is quite pure as determined by tlc and pmr analysis and was used as is for further reactions. A sample of the foam (5 g.) dissolved in ethyl acetate was passed through a short column of silica gel (50 g., packed in ethyl acetate) to provide 4.5 g. of **8** as a colorless, hard foam; ^1H nmr (DMSO-d_6): δ 5.31 (d, 1, J = 2.5 Hz, C_3H), 5.74, 5.89 (dd, 1, J = 7.5, 2.5 Hz, C_5H), 6.00 (d, 1, J = 2.0 Hz, H_1'), 6.35 (s, 2, NH_2); $[\alpha]_D^{25}$ -48.0 (*c* 1, methanol).

Anal. Calcd. for $C_{31}H_{26}N_2O_8$ (554.5): C, 67.14; H, 4.73; N, 5.05. Found: C, 66.98; H, 4.73; N, 4.95.

4-Amino-1-β-D-ribofuranosyl-2-pyridone (3-Deazacytidine, **9**).

A solution of **8** (55.4 g., 100 mmoles) and anhydrous methanol (250 ml.) was saturated with ammonia at 10° and placed in a steel bomb (300 ml.) for 24 hours. The solution was evaporated *in vacuo*, and the residue was triturated with dry ether (500 ml., in 4 portions). Recrystallization of the residue from methanol provided **9** (20.8 g., 86% in two crops) as large, light yellow cubes, m.p. 209-210° (12) (after drying at 100° for 2 hours) (Lit. 208.5-210° (4d)); $[\alpha]_D^{25°}$ + 4.33 (*c* 1, water) [(Lit. $[\alpha]_D^{29°}$ + 4.6° (*c* 1, water (4d))]; λ max (*p*H 1): 259, 218 nm (ε, 14,880; 23,410); λ max (*p*H 7): 274 sh, 261, 218 (7,670; 9,270; 29,760); ^1H nmr (DMSO-d_6): δ 5.29 (d, 1, J = 2.5 Hz, C_3H), 5.72, 5.84 (dd, 1, J = 7.5, 2.5 Hz, C_5H), 5.98 (d, 1, J = 4 Hz, H_1'), 6.15 (s, 2, NH_2), 7.55 (d, 1, J = 7.5 Hz, C_6H).

Anal. Calcd. for $C_{10}H_{14}N_2O_5$ (242.2): C, 49.58; H, 5.82; N, 11.57. Found: C, 49.32; H, 5.74; N, 11.32.

4-Hydroxy-1-β-D-ribofuranosyl-2-pyridone (3-Deazauridine, **11**).

Powdered **9** (4.84 g., 20 mmoles) was treated as described for the preparation of **5** *via* method B except that ice-bath temperatures were employed during addition of sodium nitrite and subsequent stirring was at ambient temperature for 15 minutes (temperature rose to *ca.* 20°). Recrystallization of the residue obtained after resin and charcoal treatment from methanol-acetone provided **11** (4.1 g., 85% in two crops) as colorless needles, m.p. 230-232° (after drying at 80° for 5 hours) (12); $[\alpha]_D^{25°}$ + 34.1 (*c* 1, water); λ max (*p*H 1): 278 nm (ε, 4470); λ max (*p*H 11): 255 (8280), 268 sh (6450), λ max (methanol): 282 (4720); ^1H nmr (DMSO-d_6): δ 5.98 (d, 1, J = 2.5 Hz, H_1'), 5.56 (d, 1, J = 2.5 Hz, C_3H), 5.95 (distorted q, 1, J = 2.5 Hz and J = 7.5 Hz, C_5H), 7.78 (d, 1, J = 7.5 Hz, C_6H).

Anal. Calcd. for $C_{10}H_{13}NO_6$ (243.2): C, 49.38; H, 5.39; N, 5.76. Found: C, 49.32; H, 5.38; N, 5.74.

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(1a) Author to whom correspondence should be directed; present address: Warner-Lambert/Parke-Davis Pharmaceutical Research Division, Ann Arbor, Michigan 48106; (b) Travenol Labs International, 3300 Hyland Avenue, Costa Mesa, California 92707; (c) Department of Chemistry, Brigham Young University, Provo, Utah 84602.

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